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United States Department of Agriculture

Animal and Plant Health Inspection Service

CVB Regulatory Perspective - *Toxoid Vaccines*





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Center for Veterinary Biologics

APHIS

VS Veterinary Services
Safeguarding Animal Health

NCAH National Centers for Animal Health

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Regulatory Authority

Virus-Serum-Toxin Act

- **Title 9 of the Code of Federal Regulation (9 CFR)**
  - Section 113.5: General Testing-Product released only after testing to establish pure, safe, potent and efficacious veterinary biologic
- **Veterinary Services Memorandum**
- **Center for Veterinary Biologics Notices**
- **Other Guidance Documents: Supplemental Assay Methods, Testing Protocols, Reagent Data Sheets**

  https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics
- **Product Specific: Outline of Production**
  - Section V (Final Product Testing Requirements)

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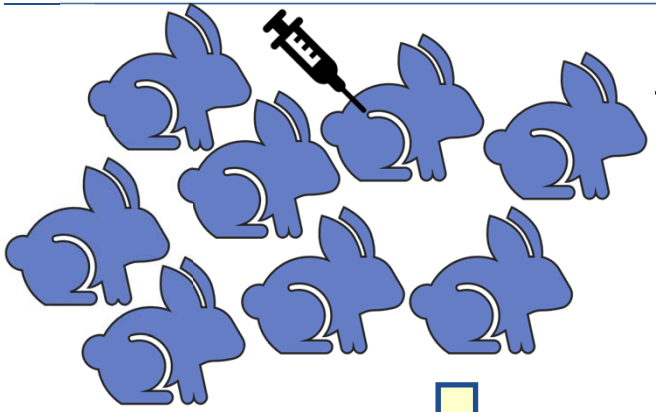
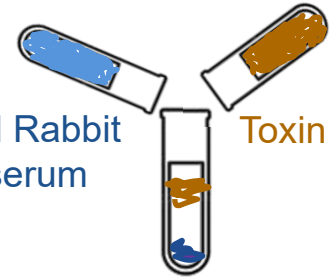
Toxoid Vaccines

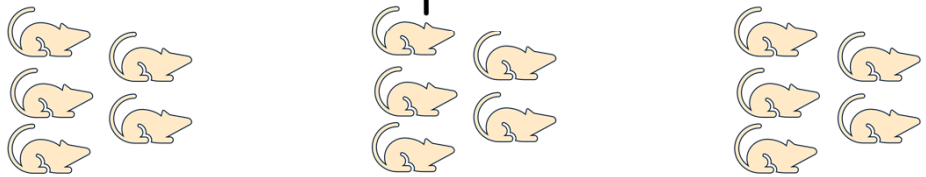


9 CFR Tests
C. perfringens Type C
C. perfringens Type D
C. sordellii
C. novyi

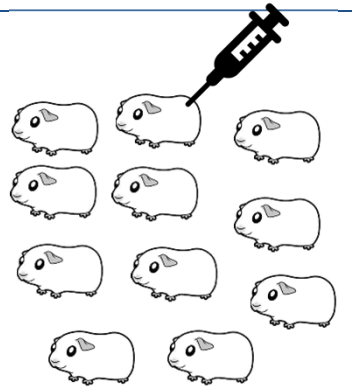
Outline/CVB Protocol
C. septicum

Wait –
Collect Serum





9 CFR Tests
C. tetani



Wait –
Collect Serum

Test Pooled
Guinea pig
~~A~~ntiserum on ELISA

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Toxoid Vaccines

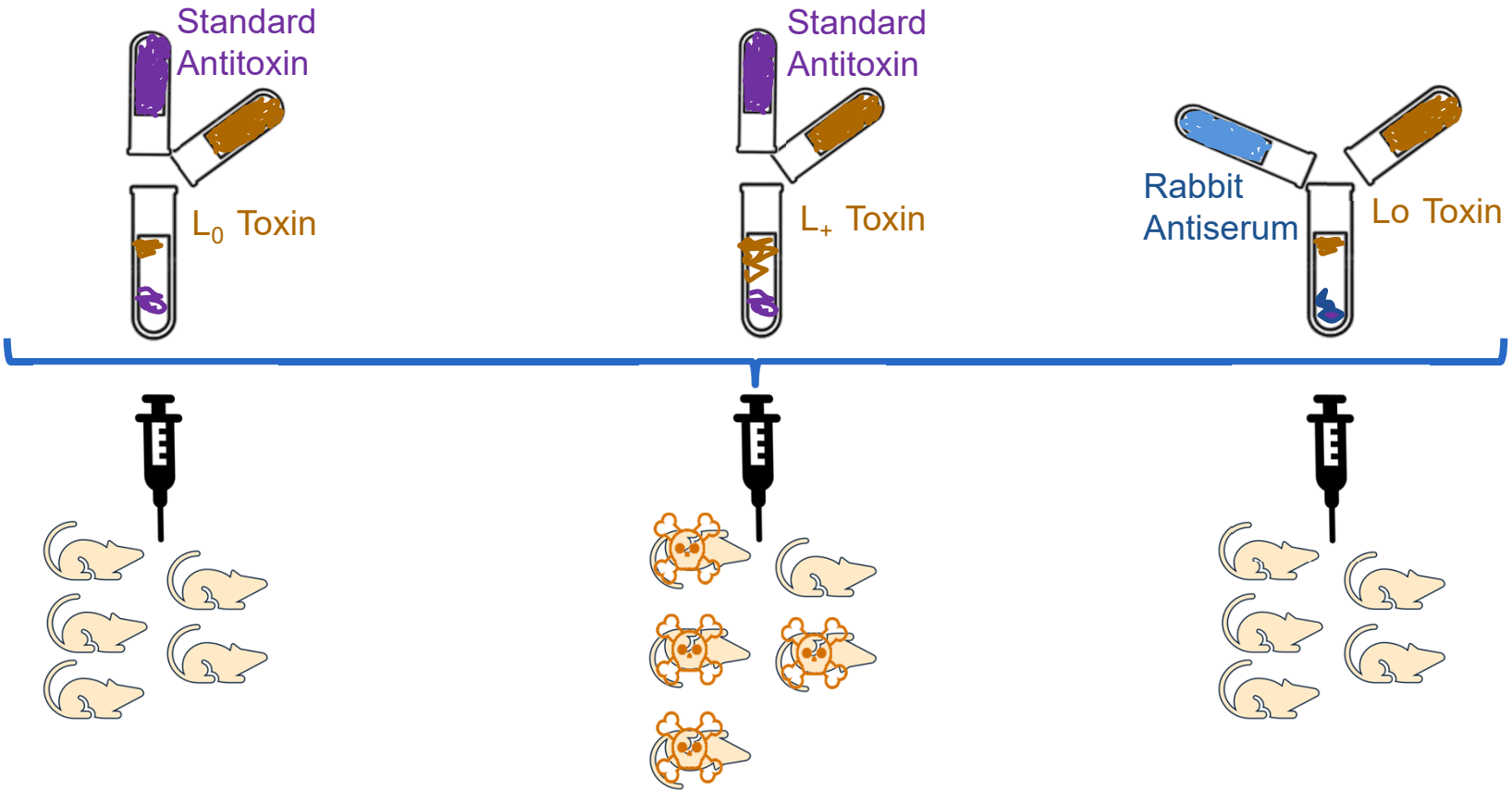
L_0

Greatest amount of toxin that, when mixed with 1 AU, results in 100% survival

L_+

Least amount of toxin that, when mixed with 1 AU, results in 80% death

Fraction	Level of Test
C perfringens Type C	10 AU/mL
C perfringens Type D	1 AU/mL
C. sordellii	1 AU/mL
C. novyi	0.1 AU/mL
C. septicum	1 AU/mL



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Toxoid Vaccines

Difference	US	EU
Requirement for batch release	See Table	
Rabbits vaccinated	8+	10+
Rabbit dose	≤ ½ Label Dose	≤ Label Dose
Mouse Inoculation Route	IV	IV or IP
Determination of units in rabbit antiserum	Lo toxin mixed w/ pooled rabbit antiserum	LD_{50} determination through titrations over time
Mice per treatment group	5	2

Release Requirement

Fraction	US	EU
C perfringens Type C	10 AU/mL	10 IU/ml
C perfringens Type D	2 AU/mL	5 IU/mL
C. novyi Type B	0.5 AU/mL	3.5 IU/mL
C. septicum	varies	2.5 IU/ml

Cell Based Toxin-Antitoxin Assays

- Potency Test for *Clostridium septicum* Alpha Antitoxin Using a Cell Assay
  - VERO Cells
  - https://www.aphis.usda.gov/animal_health/vet_biologics/publications/BBPRO1009.pdf
- Potency Test for *Clostridium perfringens* Type D epsilon Antitoxin Using a Cell Assay
  - MDCK Cells
  - https://www.aphis.usda.gov/animal_health/vet_biologics/publications/BBPRO1008.pdf
- Both add increasing amounts of toxin to a standard and unknown antitoxin to determine the amount of antitoxin present by monitoring cell death

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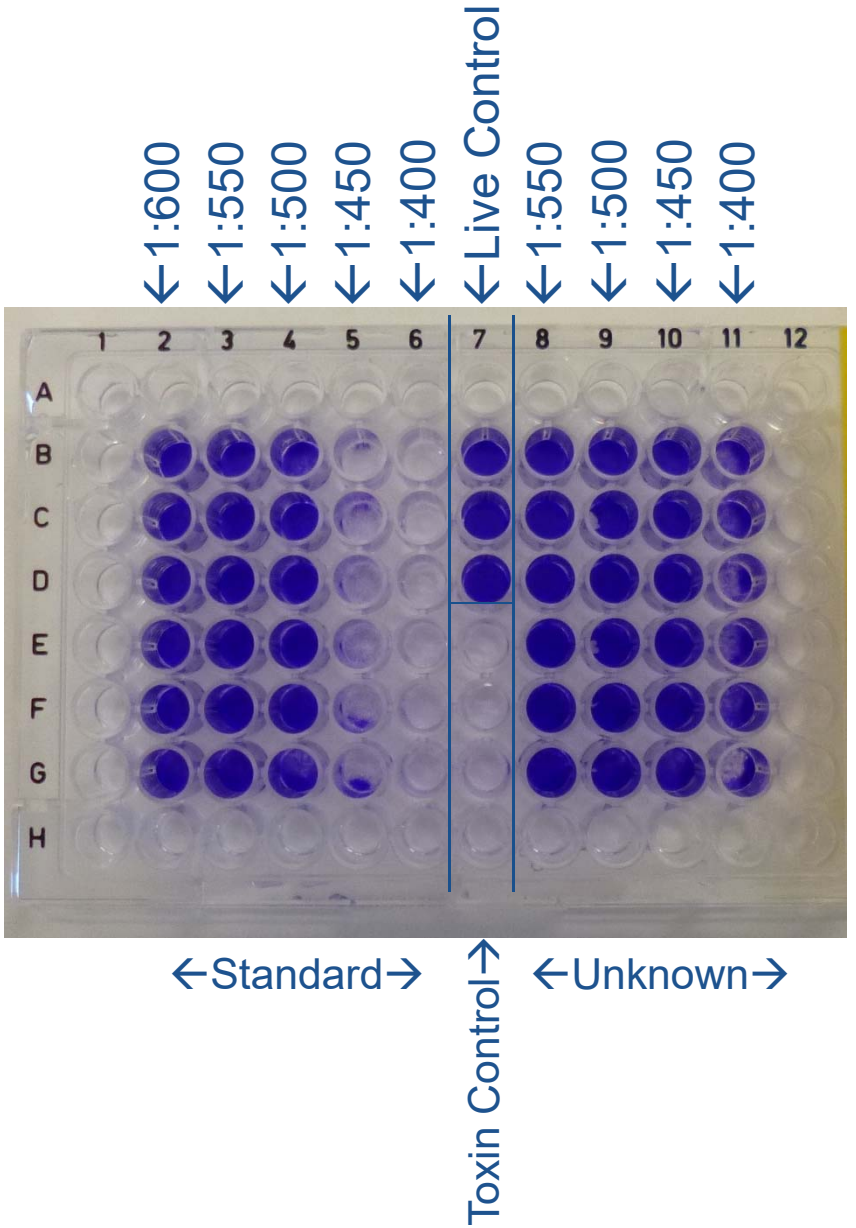
Cell Based Assays: Template

	1	2	3	4	5	6	7	8	9	10	11	12
A		Standard Antitoxin						Unknown Serum				
B		Toxin @ 1:600	Toxin @ 1:550	Toxin @ 1:500	Toxin @ 1:450	Toxin @ 1:400	Live cell Control	Toxin @ 1:550	Toxin @ 1:500	Toxin @ 1:450	Toxin @ 1:400	
C												
D												
E							Toxin Control					
F												
G												
H												

Example 1

- Satisfactory Serial:
  Cell protection for unknown serum ≥ Cell protection of Standard
  - Purple wells are “Live” or “Protected”

- Example Results:
  - Standard protects cells at 1:500 toxin dilution
  - Unknown protects cells ≤ 1:400 toxin dilution
  - Disposition: Satisfactory



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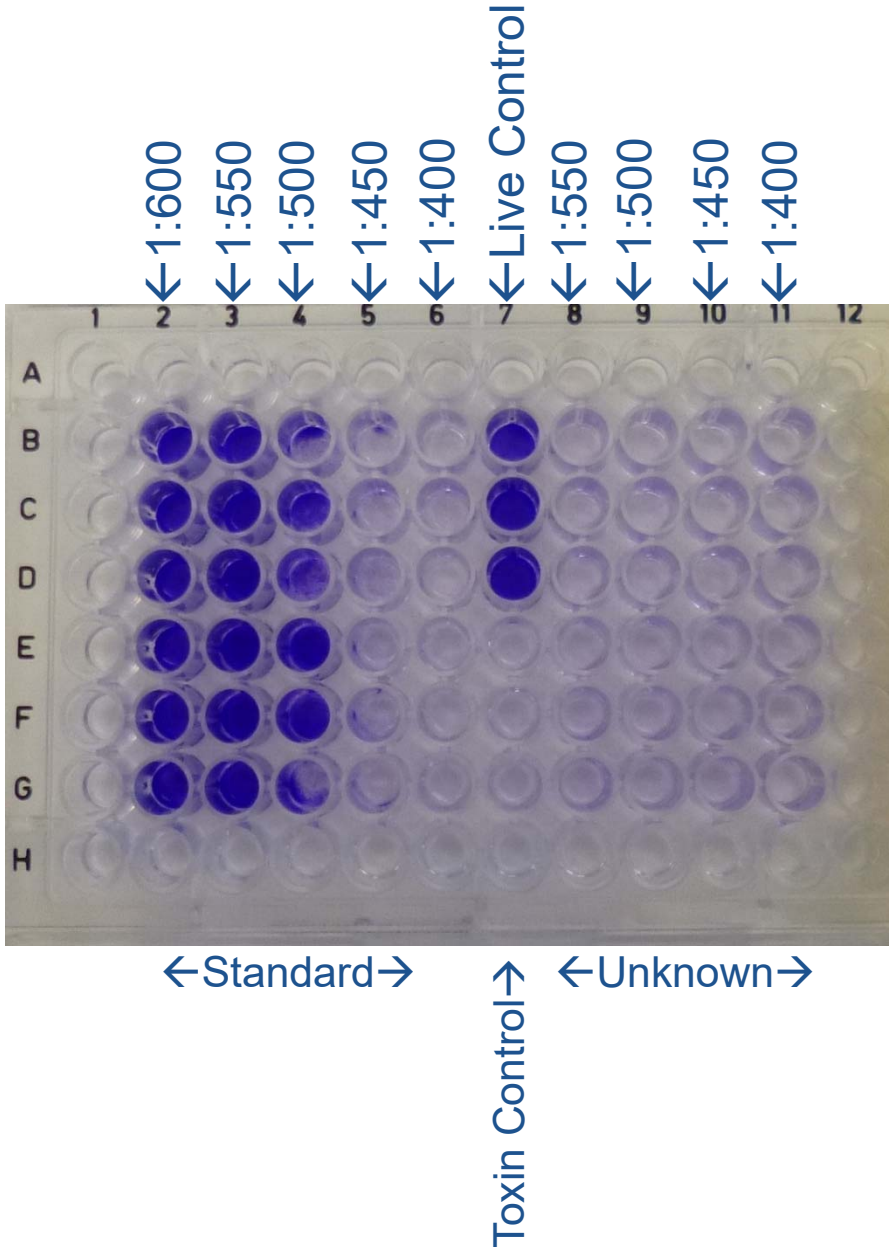
Example 2

- Satisfactory Serial:
  Cell protection for unknown serum ≥ Cell protection of Standard
  - Purple wells are “Live” or “Protected”

- Example Results:
  - Standard protects cells at 1:500 toxin dilution
  - Unknown protects cells > 1:550 toxin dilution
  - Disposition: Unsatisfactory



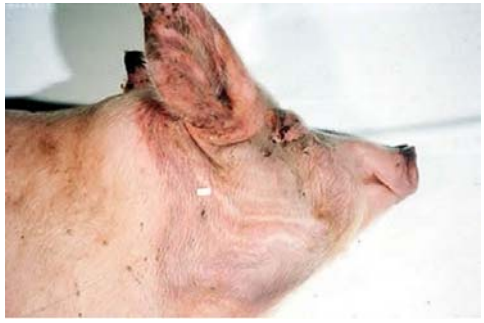
Conversion of Release Testing

- Manufacturers that wish to switch to the cell assay method will be required to do a limited product validation
- CVB provide reagents to assist with validation
  - Working stocks (conserve reagents)
  - Note in your APHIS 2018 “Use for cell assay validation”
- The validation should include the CVB-furnished proficiency panel
  - IRP 639: Clostridium septicum Cell Assay Proficiency Panel
  - IRP 638: Clostridium perfringens Type D Cell Assay Proficiency Panel
- CVB welcomes feedback on the test methods

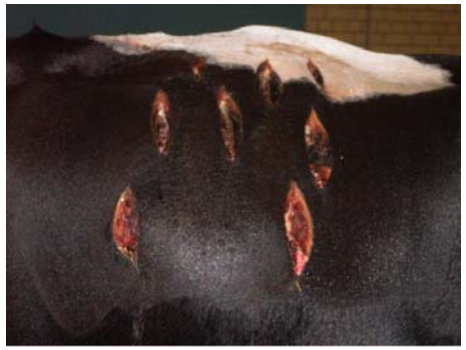
Importance of Animal Welfare

- CVB supports implementation of 3Rs in release testing.

- The goal of potency testing is to protect the animals in the field. All new assays must provide the same confidence as those currently approved in order to prevent their suffering.



C. septicum: Courtesy of the Department of Pathobiology, University of Guelph.

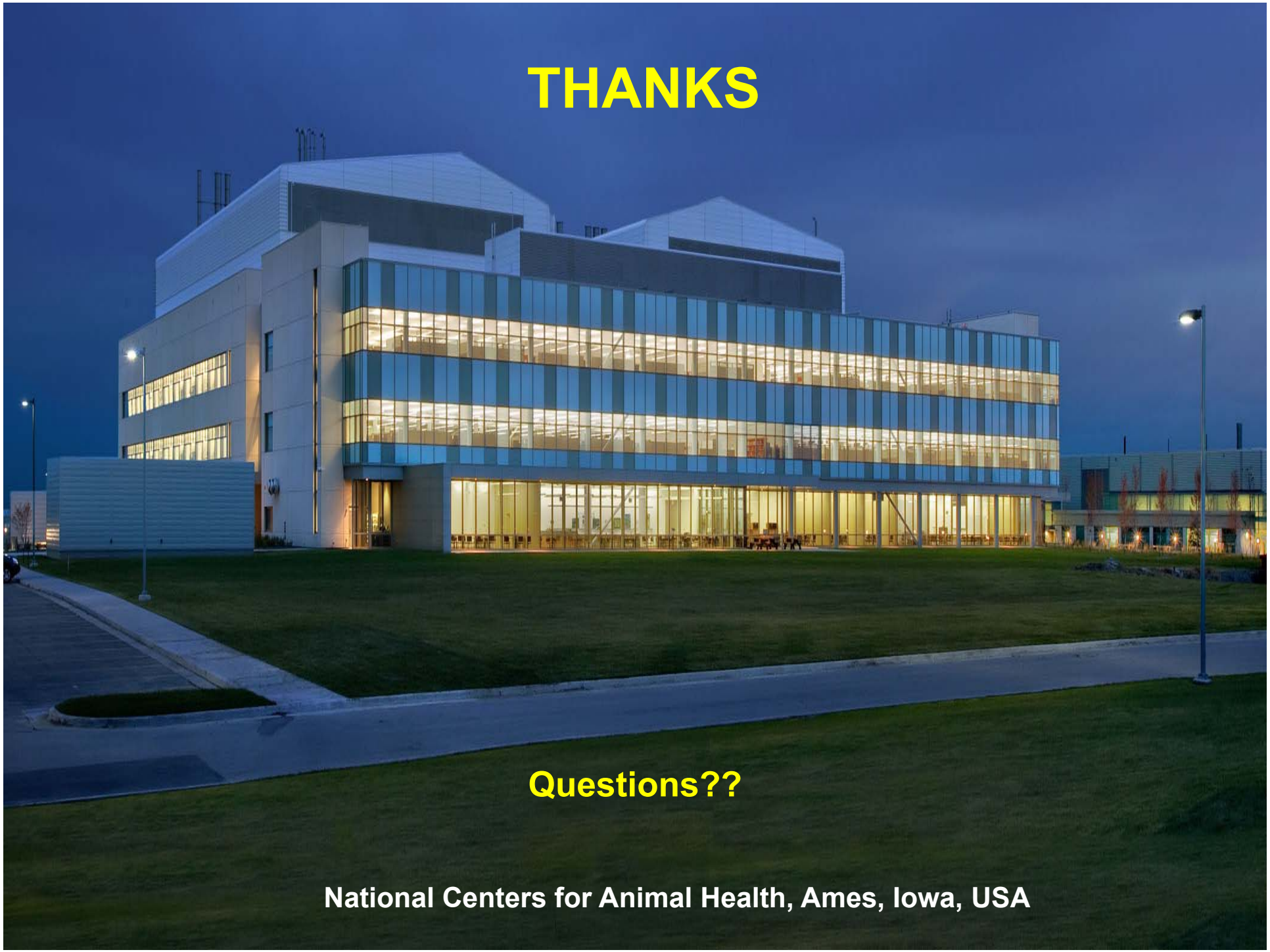


C. novyi: Courtesy of Dr. Henry Stämpfli..

https://www.merckvetmanual.com/generalized-conditions/clostridial-diseases/malignant-edema

Conclusion

- Overview of the current regulatory approach
- Guidance documents for the cell assays that have undergone proof-of-concept testing at the CVB
- Reagents
  - Available domestically
- Contacts:
  - Angela.M.Walker@usda.gov
  - Janet.M.Wilson@usda.gov


THANKS
Questions??
National Centers for Animal Health, Ames, Iowa, USA